Disposable Polymerase Chain Reaction Device

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The September 11, 2001 attack on the United States and the subsequent anthrax scare have raised antiterrorist vigilance to new heights. Although the September 11 attack was orchestrated with hijacked planes, the next threat could be nuclear, chemical, or—as evidenced by the anthrax scare—biological. Many countries that may support terrorist activities are likely to have biological weapons capabilities; biological weapons are probably also available on the black market and accessible to domestic terrorists. Planning and equipping for a biological attack requires detection devices that are robust in the field, easy to use, and relatively inexpensive. This project will leverage LLNL's competencies in microtechnology and instrumentation and will provide new capabilities in support of LLNL's homeland security mission.

To take appropriate action, first responders to a biological attack (local emergency response personnel, military, or intelligence personnel) must identify the specific organism used in the release. For this purpose, polymerase chain reaction (PCR) assays are becoming increasingly important because they can amplify a target segment of DNA (by thermal cycling of the necessary reagents) and enable identification of pathogens. However, the sophisticated tools needed to assess a potential biological release not only are unaffordable to many first-response agencies, but also require highly trained individuals.

In this project, we are designing and demonstrating a compact, disposable PCR unit based on a novel thermal cycler. The PCR device will allow first responders at the scene of a biological attack to quickly identify the nature and extent of the attack. By building on LLNL's success in designing portable PCR units, we are seeking to develop low-cost, disposable PCR units to be used by first responders. Authorities looking for the source of contamination in food or water will also benefit from this device.

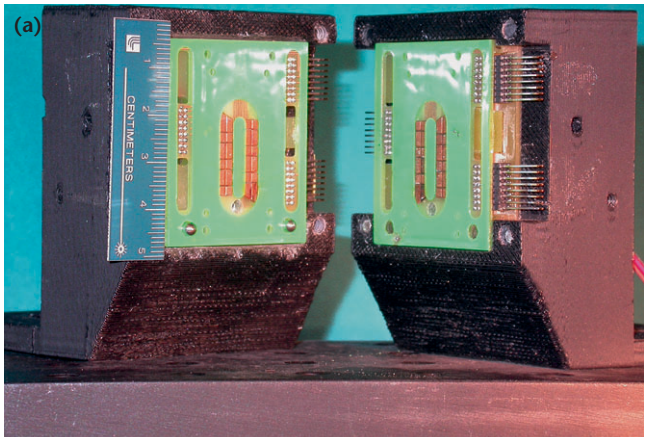
During FY02 we focused on improving and testing our novel convective PCR thermal cycler, which uses the thermal convective forces created by fixed, nonfluctuating, hotter- and cooler-temperature regions to thermally cycle the sample fluid to achieve amplification. The advantage of this approach is lower power requirements, increasing the probability that small batteries will suffice as the system's power source. We used our thermal cycler to successfully amplify a 90-base-pair, multiple-cloning site segment of a DNA plasmid—one of the first times DNA amplification has been accomplished using convective forces.

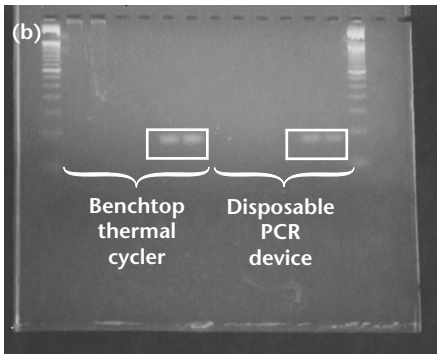
Successful DNA amplification requires an optimum temperature profile in the thermal cycler—for example, its heaters must not produce local hot spots. We fabricated several types of heating systems and achieved one that satisfied this and other thermal requirements; a patent application was filed for this heating scheme.

Sample fluid velocity was also addressed. Because the DNA amplicon—the amplified segment of DNA—in a sample fluid doubles each time the fluid cycles through the PCR unit, greater fluid velocity reduces the time needed for detectable amplification, further lowering power requirements. Measurements made with digital particle image velocimetry during test cycling yielded a fluid velocity of 1.6 to 2.4 mm/s—in excellent agreement with the velocity (2.8 mm/s) predicted as necessary with models in FY01.

In addition, we began designing a sample-preparation system based on a pillar chip—a device that captures DNA on silica pillars while inhibitors are removed, after which a series of wash steps releases the DNA from the pillars for thermal amplification.

In FY03, we will continue thermal optimization of the cycler and begin work to incorporate sample preparation with the thermal cycler—crucial for an integrated, easy-to-use, low-power, robust, and disposable PCR device.





(a) The convectively driven polymerase chain reaction (PCR) thermal cycler developed in this project. (b) Gel detection showing successful amplification of a 90-base-pair, multiple-cloning-site segment of plasmid DNA using our device, compared to amplification with a standard benchtop thermal cycler.